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Title: Controlling cellular biochemistry with electronic signals – a step towards bioelectronic hybrids

Abstract: The idea of combining man-made and natural systems has fascinated humans for centuries. However, despite the explosion of life science advances, the integration of electronic and biological systems remains woefully underdeveloped. To address this, we will apply principles of Synthetic Biology to develop biological cells controlled by electronic signals.

We propose to create synthetic cellular receptors selectively activated by electrochemically activated peptide. In order to endow the system with the ability to activate a selected ligand:receptor pair in the presence of other polypeptides, we propose to construct peptide-selective bio-electrodes. Electro activation of the peptides decreases their affinity for the electrode and leads to its relocation to the two component cellular receptor that utilizes intramolecular proteolysis to activate transcription of the reporter genes. Using the team's expertise in protein, cellular and electronic engineering we will design an integrated system where the activation of the reporter expression can be both controlled and monitored using electrode array in a few or even in a single mammalian cell.

This proposed approach enables the construction of a potentially unlimited number of orthogonal electrode/ peptide/receptor systems that allow multichannel information transfer between computing devices and biological cells. While the first embodiment relies on the relatively slow gene expression readout, the same approach can be used to control practically any biochemical process in real time. Such synthetic signaling pathways capable of performing logical operations will exert complex and precise control on cellular biology, leading to a first generation of bioelectronic hybrids.

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Title: Integrating mechanotransduction in development: how does cell shape dictate chromatin remodeling?

Abstract: Plant development and growth are linked to cellular shape changes, which are controlled by genetic programs but also by perception of environmental signals, including mechanical cues. While both genetic regulation and mechanical control of morphogenesis were studied independently, there is a need to explore how cellular shape-associated strain and stress can mechanically regulate gene expression during differentiation. In animals, mechanical stimuli are known effectors of differentiation. They involve propagation of mechanical forces through the cytoskeleton to the nucleus, leading to chromatin remodeling and modification of gene expression. Thus, nuclear envelope proteins that control nuclear shape and transmit forces to chromatin play a key role in rapid triggering of gene expression. In plants, less is known about mechanotransduction from cell surface to the nucleus.

Using a systems biology approach and an interdisciplinary network, we propose to investigate how mechanical cues affecting cellular shaping are sensed at the nuclear envelope to drive chromatin remodeling in Arabidopsis. We will sudy a unique cellular model, the single root hair in an epidermal tissue context, with well-defined morphogenetic programs linked to cytoskeleton and nuclear dynamics. We will analyze root hair formation and growth in WT and mutants affected in either root hair development or nuclear shape. Combining in vivo live imaging and micro-mechanical measurements (rheometry), we will evaluate mechanical properties of cells and nuclei during root hair development and their dependence on cytoskeleton and nuclear dynamics in relation to gene expression. We will also determine how these mechanical, structural and biological properties are modified when a controlled mechanical stress is applied to the root hair cell during development. Our data will highlight proteins involved in mechanosensing, and we will evaluate their interaction with the nuclear envelope network. Live imaging and rheometry data will be correlated to finite element modeling to estimate strain and stress in the system for predicting chromatin remodeling following cellular and nuclear shape changes.
Altogether, this will highlight the molecular networks involved in mechanosensing at the nucleo-cytoplamic interface and reveal how gene expression is robustly regulated during cellular morphogenesis in higher plants.

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Title: Protein nanocages as single molecular reactors to understand biocatalysis in crowded environments

Abstract: Enzymes catalyze molecular transformations in living systems while being pushed and squeezed by other biomacromolecules. This molecular crowding generates excluded volume effects that result in large quantitative consequences on the rates and the equilibria of enzymatic activity by molecular mechanisms that are not yet fully elucidated. To recreate these features, we propose packing tens to hundreds of enzymes within bacteriophage capsids to create nanoreactors (NRs). Our nanoreactors allow control over packing density, protein environment, and boundaries at the nanoscale. Regulation of these conditions is key for understanding in vivo enzyme behaviour, and facilitating metabolic engineering efforts. We will focus on the mevalonate (MVA) isoprenoid pathway, which, despite demanding enzymatic cascade reactions, is widely used for production of industrially useful biochemicals. Limitations may be related to the lack of proximity between partner enzymes, unfavourable interactions under molecular crowding conditions, and/or unfavourable molecular ratios between cascade enzymes. Here we pursue an integrated study to produce materials where MVA enzyme packing and stoichiometry are controlled to mimic the complexity of the intracellular environment. The kinetics of enzymes within NRs will be measured using both ensemble and single-molecule techniques. The enzymatic activity of single nanoreactors will be monitored using a combination of atomic force and fluorescence microscopies, which will allow the in situ study and manipulation of single NRs in real time to provide individualized details not accessible with averaging ensemble approaches. These studies will be supported by multiscale modelling of the crowded enzyme environment. Our experiments will reveal the optimum biophysical conditions for maximizing metabolic flux in the MVA pathway. Further, designed NRs will be assembled and assessed within living cells. Our project has both fundamental and applied aims. First, to understand how crowding affects enzymes at the nanoscale, including metabolite diffusion and protein conformational changes. Second, to improve the biosynthesis of isoprenoids in biological and industrial systems.

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Title: Probing persistence paradigms: synthetically, immunologically and ecologically

Abstract: As intracellular, obligate parasites viruses rely on a diversity of strategies to survive. Some, like most RNA viruses, cause acute “hit-and-run” infections and require large susceptible host populations. Others, mostly DNA viruses, persistently infect their hosts. Morbilliviruses, like measles virus (MV) and canine distemper virus are examples of RNA viruses that can cause persistent infections. The exceptional circumstances which converge to drive RNA virus persistence remain unclear, since these viruses need to replicate continually in vivo. Bats are evolutionary and ecologically unique mammals standing out in their ability to tolerate viral infections. We recently showed that bats host major mammalian paramyxoviruses and described an ancient morbillivirus in vampire bats. This discovery provides a unique opportunity to explore RNA virus persistence in an important reservoir host that has high rates of contact with humans and domestic animals through its blood feeding habits. The primary objective of the proposed research is to unravel fundamental mechanisms that govern morbillivirus persistence and understand the consequences for immunity and infection. We hypothesize that within-host persistence in canonical morbilliviruses is a vestigial relic of a more common strategy that evolved in bats. Research activities comprise a multidisciplinary program involving investigations of natural and experimental infection of bats, reverse genetics to resurrect viruses from infectious clones, comparative studies of canonical morbilliviruses and hosts together with investigation of pathobiology and immunity underpinned by mathematical modeling. Existing materials, a unique field network for long-term monitoring vampire bats, novel approaches and critical insights from systematic field and laboratory studies make this project both exciting and feasible. This study represents an unusual integration of approaches and ideas from disease ecology, evolutionary biology and synthetic biology. We expect this fusion to resolve fundamental knowledge gaps in our understanding of how RNA viruses persist in hosts; a critical component for the understanding of rare disease manifestations, the consequences of viral persistence on the host immune system and for identifying patterns in risk of virus transmission between species.

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Title: Handling OXPHOS structural heterogeneity and metabolic plasticity

Abstract: The OXPHOS system is the only process in animal cells with components encoded by two genomes, maternally transmitted mitochondrial DNA (mtDNA) and biparentally transmitted nuclear DNA (nDNA). The protein products of both genomes have to physically assemble with their counterparts to build functional respiratory complexes. Therefore, variability in the OXPHOS encoded genes is limited by a physical match constraint. This imposes a close-fitting co-evolution of both genomes challenged by the very different mechanism to generate variability for nDNA (by sexual reproduction, mutation and co-existence of two alleles) and mtDNA encoded OXPHOS genes (by mutation, polyploidy and segregation). Since the simultaneous co-existence of alternative mtDNA encoded alleles for OXPHOS proteins has been shown to be detrimental for the organism, we postulate that the co-expression of nuclear encoded alternative alleles may have similar adverse consequences, and that specific regulatory mechanism prevent them. Indeed, random mono-allelic expression is not rare and was suggested for about 30 OXPHOS genes in mice. We postulate that is part of a sophisticated and multi-level system of quality control and functional testing to select for the best combination for providing metabolic plasticity.
Potential regulatory mechanisms are: selective transcription of one allele per cell or selection of the expressed alleles at the import or assembly of the OXPHOS complexes. For testing this hypothesis, we integrate different skills for the analysis of mitochondrial functional and genetics profiling (Enriquez), single cell transcriptomic (Eberwine) and functional and dynamic analysis (Busch) in mouse (Enriquez) and zebrafish (Mercader). If confirmed, we will set the ground for a novel theory of genetic interaction for OXPHOS function. If discarded, the existence of mtDNA and its particular way of inheritance would be necessary to group those genes for which allelic variability is detrimental.

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Title: Sleep, the clock, and the brain: a neuromathematical approach

Abstract: One of the most basic aspects of sleep is that it happens at a particular time of day. Neuroscientists have known for almost half a century that this consolidation requires the suprachiasmatic nuclei of the hypothalamus (SCN); beyond this point, the circuit remains untraced. Equally mysterious, healthy young humans sleep in a single consolidated bout, while infants and older individuals, as well as laboratory mice, can have highly fragmented sleep. Informed by a combined cellular and circuit-based model for slow-wave or "deep" sleep (Forger Group), we propose to trace the signals leading from SCN to cortical slow waves at both physical and molecular levels, and then manipulate them to artificially consolidate sleep. Starting from cortex, aided by a novel "triple-CRISPR" approach to generate knockout mice efficiently in a single generation, we shall examine roles of individual cortical ion channels and the signaling pathways they regulate in creating these slow waves both in vivo and in newly developed whole-brain culture (Ueda Group). This information will be incorporated into a cellular and then a thalamocortical model of slow-wave sleep (Forger Group). In parallel, synaptic tracing in cleared brain, as well as calcium imaging correlation analysis using miniature skull-mounted microscopes, will establish physical and functional connectivity from SCN to cortex (Brown Group), which can also be tested in whole-brain culture (Ueda group). Modeling these data comprehensively and then optimizing this whole-brain model (Forger group), we can predict and test molecular sources of homeostatic and circadian influence upon sleep, as well as combined transgenic and optogenetic strategies to create consolidated bouts of sleep from the normally fragmented sleep of the mouse (Brown and Ueda groups). In this way, by using the power of large-scale quantitative modeling to explore synergies in sleep-dependent signaling, we hope to provide a starting point for novel multimodal therapies with the capacity to fundamentally alter the sleep-wake landscape.

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Title: Quantitative dissection of molecular determinants of enhancer function

Abstract: Enhancers are relatively short DNA sequence elements (<1 kb) that determine the timing, location and levels of gene transcription. They harbor specific binding sites for transcription factors (TFs) that control the enhancer. While the necessity of particular TF binding sites (TFBSs) can be assayed with mutations, it is not yet understood which DNA features in an enhancer sequence collectively give rise to the regulatory activity. To identify the molecular determinants that impart a regulatory activity to a DNA sequence element, we have devised a quantitative experimental paradigm and propose to apply statistical analysis and machine learning approaches to the molecular dissection of a model enhancer.

Specifically, we will use a model enhancer driving patterned spatial expression in the wings of fruit flies (Drosophila). First, we will create tens of variants of this enhancer, introducing mutations along its sequence, and describe their regulatory effect. To this end, we will build an automated imaging pipeline to measure the levels and spatial distribution of reporter gene in the wings. The resulting quantitative expression data in a flat tissue will define a morphospace, i.e., a mathematical multidimensional space representing the possible variation of enhancer activity. Second, we will extract DNA feature sets to quantitatively describe molecular variation along the sequence of our mutant enhancers. This will capture structural changes (DNA shape readout), changes in nucleotide sequence per se (DNA base readout), as well as other features (e.g., TFBSs). Third, leaning on dimensionality reduction techniques and machine learning, we will develop predictive models that describe the relationship between DNA features and morphospace. Finally, we will test our predictions of enhancer functionality using synthetic enhancers in transgenic flies. Because enhancers are complex biological objects, we aim with this proposal at developing appropriate mathematical tools to capture the essence of this complexity.

Since this proposal aims at developing a comprehensive mathematical approach to modeling of enhancer functionality, it has the potential to unravel complex molecular mechanisms underlying transcriptional regulation.

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Title: From molecular stochasticity to robust cell divisions

Abstract: Cells achieve reproducible outputs while relying on intrinsically stochastic molecular processes. In plants, cell division orientation is accurately predicted before mitosis by a microtubular ring, the preprophase band (PPB), through an unknown mechanism. Using high-throughput microscopy, quantitative image analysis, biomechanics and stochastic modeling, we will investigate how the stochasticity of microtubule (MT) self-organization is used, or filtered out, during PPB formation to sense temporal, geometric and mechanical cues in order to generate a robust placement of cell division planes. In short, we will test whether the PPB acts as a macromolecular mechanosensor. Using statistical mechanics such as Monte Carlo, event-based modeling and mean-field theory, we will assess how stochasticity leads to distinct dynamical states for MT arrays. We will develop biophysical models of dynamic MTs in 3D cells to explore how stochastic MTs self-organize into PPBs and how spatiotemporal cues modulate that process. Using the Arabidopsis shoot apical meristem, we will identify correlations between MT array dynamics, PPB behavior, cell shape, growth and mechanics, cell cycle progression, and cell division plane from statistically representative sample involving hundreds of dividing cells. To challenge the robustness of PPB and cell division in vivo and in silico, we will globally and locally increase variability in these cell parameters with mutants (MT dynamics, wall mechanics, cell cycle, mechanotransduction), inducible lines, mosaics, and micromechanical perturbations. Last, we will unravel the molecular mechanism processing molecular stochasticity to channel cell divisions: we will investigate how the TTP (TON1-TRM-PP2A) complex, a key regulator of PPB formation in connection with the cell cycle, contributes to generate reproducible divisions by monitoring MT self-organization, integrating geometric, mechanical and temporal cues. Implications of this project go beyond cell division robustness: while many cellular pathways are adapted to respond to rapid and discontinuous changes, we expect here to unravel mechanisms managing slow and continuous signals, like shape, growth or tissue-stress. We will also gain insight into the mathematical properties of stochastic extended objects like microtubules, and we will propose a mechanism for cells to perceive directional cues.

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Title: New letters to the DNA alphabet

Abstract: The recent discovery of 7-deazapurine bases in bacterial and bacteriophage DNA revealed an unexpected interplay between RNA and DNA modification pathways. In phages, these hypermodified bases could play major roles in pathogen-host interactions, DNA replication, DNA-packaging into capsids, gene expression and bacteriophage evolution. Using a combination of genetic, biochemical and sequencing approaches, our objectives are to discover i) how widespread is this phenomenon, ii) the exact pathway(s) leading to the incorporation of these alternative bases into DNA, iii) the "raison d'être" for these modified bases, and iv) if this phenomenon arose as an offensive or as a defensive tool.

To characterize the distribution of 7-deazapurine bases in bacteriophages DNA, we will use several approaches including comparative genomics, mass-spectrometry analysis of modified bases, high-throughput sequencing of restriction endonuclease resistant environmental virome samples, and genome sequencing of phages infecting a wide range of bacterial hosts. This will generate a collection of diverse, sequenced and partially characterized phages that will be made publicly available via deposition in the Félix d'Hérelle Reference Center for Bacterial Viruses. Biochemical, molecular biology and genetic studies will be performed on phages harboring a variety 7-deazapurine variants to elucidate these novel DNA modification pathways. We will also study the role(s) played by the DNA modifications in phage biology, with a particular emphasis on understanding their potential role in overcoming host defences or in facilitating phage-mediated destruction of unmodified host DNA. In addition to classical biochemical, genetic and omics methods, third generation sequencing (PacBio and Nanopore) technologies will be exploited. These can already detect simple DNA modifications, like methylation, but we will combine machine learning with the sequencing of phage DNA molecules containing specific 7-deazapurines to expand the range of detectable DNA modifications. Although the scope of this project is limited to deciphering the role of 7-deazapurines in bacteriophage DNA, our results could spark developments of new genome editing tools and increased knowledge on gene regulation and epigenetics that may some day change paradigms in human health.

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Title: The architecture of the postsynaptic density

Abstract: The human brain gains much of its computational abilities from the trillions of connections made between cells by synapses. Molecular changes in synapses (a process called synaptic plasticity) are considered to underlie learning and memory. An important component of the synapse is the postsynaptic density (PSD). This specialized structure has been studied extensively to understand its function, in particular because >100 neurologic disorders (such as autism spectrum disorder and schizophrenia) have been associated with PSD dysfunction. Information regarding the overall molecular architecture of the PSD, however, is largely incomplete, in part because the PSD is extremely complex, containing hundreds of individual components connected in a dense network. The PSD is also highly dynamic and asymmetrical—two properties that render protein structural analyses challenging. While solving the structure of the entire PSD seems insurmountable, half of the PSD's mass is composed of only ten classes of linker proteins. We hypothesize that these highly-abundant proteins form a molecular scaffold, a network we term the 'postsynaptic scaffold' (PSS). We aim to develop an approach we call the 'Thermophile-Assisted Postsynaptic Architecture Strategy' (TAPAS) to solve the PSS structure. Given the delicate nature of proteins, structural biologists like to work with resilient, temperature-resistant proteins that can be obtained from thermophilic organisms. For brain-derived proteins, there is one animal known to be comfortable at temperatures >50 °C: a worm that lives on hydrothermal vents in the Pacific Ocean. We plan to sequence this worm's genome (Alvinella pompejana) to find the PSS proteins that it shares with humans. We will determine the structures of the thermophilic proteins by X-ray crystallography. As much of the PSS architecture consists of filamentous structures that do not crystallize, we will use cryo-electron microscopy to define these larger structures. Finally, we will integrate several other methods (biochemical, proteomic, bioinformatic and advanced electron tomography methods) to build a model of the overall PSS. Achieving this ambitious goal will inform studies into learning and memory, lead to new treatments for devastating brain disorders, and help explain how the synapse contributes to human cognition.

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Title: Can evolution minimize spurious signaling crosstalk to reach optimal performance?

Abstract: The complexity of cellular machineries has been forged by the motors of evolution (mutation, natural selection, and genetic drift), with boundaries that are determined by the properties of biological molecules and the biophysical limits of the cell. Our goal in this project is to determine what are the relative contributions of biophysics and evolution in shaping this complexity. Our model of interest is cell signaling, which is rapidly evolving and involves highly interconnected networks of proteins that sense and process signals and make real-time decisions to satisfy cellular needs and promote survival and proliferation. Because typical cells contain from millions to billions of protein molecules, signals can reach out to unwanted proteins in an unspecific manner. How much can evolutionary forces optimize this process so that cells can reach biophysical optimality by minimizing unspecific interactions is currently unknown. This question can be answered neither from physics nor from biology alone, and thus requires a multidisciplinary team. Our international team with expertise in theoretical physics (Gasper Tkacik), evolutionary biology (Christian R Landry) and biochemistry (Judit Villen) is poised address this question. We will generate a mathematical model of signaling network evolution that includes key biophysical and evolutionary driving forces to examine how each contributes to create unspecific signaling interactions in organisms with finite population sizes. This model will guide experiments in which we will manipulate the amount and extent of unspecific interactions and measure its biochemical impacts and its effects on fitness using experiments and bioinformatics data integration to calibrate parameters of the model. In biophysics, it is often assumed that biological systems have reached an optimum attained by natural selection and are only limited by their physical characteristics. Evolutionary biology considers that natural selection works against genetic drift and mutations to bring biological networks near their optimum, which is unknown. Our work between these two fields will reveal how close to the biophysical limits can signaling networks get and how biophysical and evolutionary forces are integrated to shape accurate signal transduction.

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Title: Dynamics of collective cell migration on curved surfaces

Abstract: Epithelial collective cell migration (CCM) is important in biological processes such as organ development, tissue maintenance and tissue repair. CCM usually occurs on curved surfaces such as that found in blood vessel walls and intestinal villi. However, current CCM research are still conducted on flat surfaces. This is due to lack of techniques to fabricate tubular and spherical microstructures resembling that of organs as well as difficulty in observing and measuring the collective dynamic behaviour of cells on these surfaces. Nevertheless, recent studies are beginning to show that cells do behave differently on curved surfaces. However, these studies were mainly focused on single cells, as such, there is limited insight into CCM which involves complex interactions among multiple cells.

Here, we claim that curved surfaces can significantly influence CCM. As such, we propose to systematically study CCM on such surfaces to better understand their underlying mechanisms. Our project will combine distinct but complementary scientific expertises coming from biology, biophysics and engineering. We aim to: 1) Develop novel techniques in fabricating different, well-defined curved geometries such as tubular and spherical surfaces with nano-patterns; 2) Identify biomolecules and proteins involved in CCM on curved surfaces; 3) Develop computational models to perform 3D imaging and analysis so as to better monitor and measure complex tissue dynamics; and 4) Develop a universal theory to better explain and unify experimental observations.

Together, the unique combination of these different approaches will enable us to better understand the fundamental mechanisms and impact of surface curvature on cell behaviour and tissue organization. These will shed light on related biological functions such as in organ development and tissue repair, as well as contributing towards better tissue engineering or regenerative medicine applications.

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Title: Fusion of evidence and expectation: untangling stimulus and prior information in the visual cortex

Abstract: Perceiving and interpreting our sensory environment feels subjectively effortless, yet, it is one of the computationally most challenging tasks the brain needs to solve. Critically, our brain constantly fuses current sensory input with expectations derived from prior experience in order to be able to reliably interpret our environment in spite of ever-changing conditions, such as lighting, pose, or configuration, making every single experience unique. That prior expectations are constantly shaping our percepts has been well known for over a century from behavioral studies, but how neural circuits in sensory areas of the brain achieve this sensory-prior fusion has remained largely unknown. Progress in several research fields make it possible now to address the neural bases of sensory-prior fusion: novel recording techniques provide an unprecedented opportunity to track the activity of large populations of neurons over extended periods of time; advances in artificial intelligence and machine learning provide powerful new tools to analyse complex data; and advances in computational theories allow us to formulate experimentally testable hypotheses and compare their predictions to data. These techniques are now being used to great effect in sensory neuroscience, but almost exclusively to characterise how sensory input alone impacts neural responses along different stages of the visual processing system of the brain. Our project aims to bring these advances to bear on understanding the effects of prior expectations on neural responses, and their fusion with sensory information. We will rely on complementary approaches that provide insights into different aspects of sensory-prior fusion: experts in mouse experiments and monkey experiments will train complex tasks and record from brain areas that contribute to sensory-prior fusion, while experts in machine learning-based data analysis and computational neuroscience will develop cutting-edge analysis techniques and models to guide experiments and interpret data in a formal theoretical framework. Our project will thus help us understand how a brain that is composed of unreliable components and prone to a variety of perceptual distortions and illusions can efficiently process rapidly changing, cluttered, and noisy scenes to achieve feats such as driving us home safely in busy traffic.

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Title: Structure and biophysics of disordered domains mediating RNP granules: from atoms to cells

Abstract: Ribonucleoprotein (RNP) granules are "membraneless organelles" found in eukaryotic cells. Common to RNP granules is enrichment of RNA-binding proteins harboring intrinsically disordered domains of low sequence complexity (LC) that may self interact, stabilizing granules via liquid-liquid phase separation (LLPS). Importantly, several LC domains of stress granule (SG) proteins are aggregation-prone and associated with inclusions observed in many neurodegenerative diseases. Although the constituent proteins and RNA are being enumerated by mass spectroscopy and sequencing approaches, and the ~100 nm-scale features are being resolved by fluorescence microscopy techniques with improving resolution, a fundamental conformational/physical-chemical understanding of structures and protein-protein interactions stabilizing SGs remains to be directly interrogated. Our goal is to observe with, residue-by-residue detail, the protein-protein interactions and structural changes caused by disease-causing missense mutations in human SGs both in vitro and in cells. Using the well-studied protein FUS to benchmark our spectroscopic tools and the SG marker and nucleator TIA-1 as a focal point, we will provide the first analysis of protein secondary structures and stabilizing interactions in granules. We will apply in-cell vibrational and Förster resonance energy transfer (FRET) imaging combined with NMR spectroscopy to determine the molecular details of LC domain structure in LLPS assemblies in vitro and SGs in cells. By examining disease-associated variants known to form intracellular aggregates or impair SG dynamics, we will probe the underlying physico-chemical protein changes underlying disease-associated SG biophysical changes. Our novel molecular toolbox and experimental protocols to probe protein structure in cellular (and in vitro) SGs will be immediately applicable to other RNP granules and phase separated structures and their disease-associated changes.

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Title: Integrated view of photosynthetic control in algae in response to light- and metabolic-signals

Abstract: Life on earth depends upon the photosynthetic conversion of H2O, CO2 and light into fixed carbon (C) compounds including polysaccharides and lipids, and yet the biosynthesis, regulation and many aspects of the function of the photosynthetic apparatus are still not well understood. A major challenge for photosynthetic organisms is to capture light energy for growth while at the same time eliminating excess absorbed excitation to prevent photodamage, which can be energetically costly and potentially lethal. A major photoprotective mechanism in plants and most algae is qE (quenching energy), a quenching mechanism that dissipates absorbed excitation energy as heat, ensuring survival even under adverse conditions. In the green alga Chlamydomonas reinhardtii (Cr), two LHCSR proteins (Light Harvesting Complex Stress Related; LHCSR1 and LHCSR3) are required for qE function.
The LHCSRs are nucleus encoded, and their expression is governed by transcriptional and post-transcriptional processes that are impacted by CO2, nutrient availability, photosynthetic electron transport (PET), blue light perception and calcium(Ca2+)-elicited signalling. Despite their fundamental importance, mechanisms that regulate LHCSR accumulation are still not well understood and complicated by multiple inputs. For example, our findings demonstrate that LHCSR control is impacted by the metabolism of fixed C compounds. The combined use of physiology, biochemistry, genomics and mathematical modeling will allow us to elucidate transcriptional, post-transcriptional and epigenetic processes that link photosynthesis, C metabolism and photoperception to qE activity, and help establish a systems level view of photosynthetic dynamics.

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Title: Nanoscale heat transfer phenomena: new paradigm for intra- and intercellular signalling and shaping

Abstract: Heat produced by living species has been known for centuries but viewed merely as a factor contributing to body temperature. Recent progress in ultra-local thermometry at a cellular scale has challenged this concept. Reports on heat release by active ion channels and pumps and other pioneering experiments and theoretical estimates have fuelled discussion about the significance and the magnitude of endogenous heat. Discoveries of the faster neurite growth aligned with a thermal gradient and heat-pulse induced muscle contractions without Ca2+ influx suggest that cell hot spots may channel signalling and thus contribute to cell and tissue differentiation. We will study the role of heat in three-dimensional cell shaping and differentiation and test the hypothesis that localized heat signals can affect morphology in cells other than neurons. We will aim to resolve the current controversy of intracellular thermometry, the drastic mismatch between theoretical estimates based on near-equilibrium thermodynamics and reported experimental values of temperature rise. We will investigate the signalling activity of artificial hot spots introduced into or close to cells and measure thermal activity of natural hot spots on cell membranes. We will develop a reliable intracellular nanothermometry system and an ultra-local heat-shock trigger-and-measure method to create and control the ultra-local hot spots with light. The method will use a synthetic complex of several nanoparticles, one as a heater and the others as temperature sensors. For the heater, we will use a gold nanorod whose heating can be controlled by the wavelength and polarization of light focussed on the rod. The temperature will also be measured optically, with nano-sized crystals of diamond doped with photoluminescent defects (nitrogen-vacancy centres being the primary candidate fro this role). The expertise of the applicants provides the required synergy between advanced nanotechnology, biophysics and cell biology to achieve these goals. The project starts simultaneously at four nodes and efforts will increasingly integrate as the investigation progresses.

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Title: Coupling of cell polarization and differentiation in organoids

Abstract: Understanding how out of single cells functional tissues and organs develop is a major challenge of biology. Recent progress allows us to grow organ-like cell assemblies (organoids) from stem cells in vitro. Organoids offer great potential for studying diseases and development. However, in many cases we do not yet understand how these complex tissues emerge out of progenitor stem cells. A common feature in the initial growth phase of many organoid systems is the formation of a polarized epithelial cyst with a single or multiple internal apical lumen. This initial transition into an epithelial cyst establishes a tissue template that on the one hand enables maintenance of progenitor/stem cells (niche) and on the other hand guides the patterning of differentiated cells into a functional tissue. Our aim is to understand how the interplay between proliferation (cell divisions), polarization (epithelial transition) and differentiation (patterning) leads to self-organization of this epithelial progenitor template and how this structure facilitates correct patterning into functional organoids. To this end, we will systematically control and characterize the early growth phase of two organoid systems (pancreatic and neural tube) using microfabrication and micro-patterning approaches. We will quantify evolution of cell shapes, adhesion and cortical forces, apical-basal polarization and differentiation as a function of initial cell contact patterns. This approach will provide the means to find rules how local cell interactions (cell-cell, cell-matrix, cell-lumen) are connected to tissue growth and differentiation. We will then test sufficiency of the hypothetical rules to generate the observed organoid structures using an in silico mechano-chemical model. Taken together, by dissecting the early growth phase of two organoid systems, we aim to uncover the common rules on how progenitors establish a polarized epithelial template, and how this template is then differentially used to generate organ specific differentiation patterns.

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Title: Muscle building: dissecting tension-driven myofibrillogenesis in vitro, in vivo and in silico

Abstract: Muscles are producing the active forces that enable all our voluntary body movements. The cellular components of muscle are called muscle fibers and are very large cells that can be several centimeters in length in humans. Inside all muscle cells are millions of tiny molecular machines, called sarcomeres that produce the active forces of muscles. These sarcomeres are arrayed in highly regular long chains called myofibrils that span from one muscle end to the other. This arrangement ensures that the force produced by the myofibrils is transmitted to the connected tendons and bones at the muscles ends. The highly regular periodic organization of the myofibrils results in the cross-striated appearance of skeletal muscles under the microscope.
We assembled an international team of researchers from France, USA and Germany to study how myofibrils are built during muscle development. For this we combine two different experimental model systems, the fruit fly Drosophila muscles and human muscle cells generated in vitro from stem cells, together with mathematical modeling of myofibril assembly. Our starting hypothesis is that mechanical forces, which are generated early during muscle development, are an important factor for the polymerization of myofibrils. We will test this hypothesis in fly as well as in human muscles produced in vitro by following the localization of sarcomeric protein components with high-resolution microscopy techniques. We will measure when a sarcomeric protein pattern can be first detected and how this pattern is refined. These data will be used to generate a computer model that can produce periodic sarcomere-like structures that are linked together from homogenous components. We will further measure the strength of mechanical forces present during myofibril formation in fly muscles in vivo and in human muscles in culture. Finally, we aim to modify these forces and to observe the effect of these manipulations on the developing muscles and myofibrils. These data will be used to produce a refined computer model of myofibrillogenesis. Together, our research should lead to a better understanding of how the contractile apparatus of human muscle is built, which could eventually be helpful to treat muscle injuries or aged dependent myopathies.

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Title: Evolutionary mechanics of adhesion complexes

Abstract: Basic biological processes such as cell migration, tissue morphogenesis, and hearing rely on mechanotransduction, i.e., the conversion of mechanical forces into electrical and biochemical signals that can be used by individual cells or multicellular organisms to survive. Adhesion complexes bearing mechanical force are often needed in mechanotransduction, with several protein families that include integrins and cadherins directly transmitting force signals to sites of transduction. Classical cadherins, key for tissue morphogenesis and integrity, provide calcium-dependent bonds involved in cell-cell contact formation, in signaling to the actomyosin cytoskeleton, and in mechanical stabilization of cell-cell adhesion. Non-classical members of the family are also involved in mechanotransduction, with cadherin-23 and protocadherin-15 forming an adhesive “tip link” essential for the vertebrate senses of hearing and balance. Multiple structural and biophysical studies have elucidated the unbinding, unbending, and unfolding strength of single molecules and adhesive complexes, including those formed by cadherins. However, to the best of our knowledge, nothing is known about how the mechanical properties of adhesion complexes have evolved. Here we aim to study the mechanical evolution of adhesion complexes using inner-ear cadherin tip links as a model system. Tip links initiate sensory perception by directly conveying tension to inner-ear mechanotransduction channels on a microsecond time scale. Fast and effective transmission of mechanical force must be one of the selective factors that dictate the molecular evolution of tip links. We will sequence, synthesize, and study tip-link proteins from diverse extant species and will carry out evolutionary analyses both to reconstruct the sequences of ancestral, “resurrected” tip links, and to identify potentially stronger tip links from species in which positive selection is reported. In parallel, we will use biophysical techniques to determine equilibrium binding affinities and structural properties of modern tip links from various species and ancestral tip links at different evolutionary stages. High-speed force spectroscopy and molecular dynamics simulations will be combined to probe tip link mechanics and strength across species and during evolution. Our interdisciplinary work will provide a unique evolutionary view of adhesion mechanics.

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Title: Mechanisms of chromatin reprogramming to totipotency

Abstract: The aim of this proposal is to gain insight into the mechanisms of chromatin reprogramming to totipotency in mammals. Totipotency is the potential of a cell to generate all cell types of an organism including extraembryonic tissues. Whilst induced reprogramming to pluripotency by the Yamanaka factors is relatively inefficient and takes days to weeks, natural reprogramming to totipotency occurs within hours in the one-cell embryo or zygote. How zygotic reprogramming is achieved is poorly understood. We will approach this fundamental question by studying the chromatin state of totipotent cells and their precursors. We will determine how higher-order chromatin organization changes during epigenetic reprogramming in zygotes and primordial germ cells, using both mouse embryos and human/mouse primordial germ cell-like cells. We will use our understanding of the zygotic chromatin state to identify candidate pioneer factors and to test their role in promoting totipotency and genome activation. The results of the project will provide insights into how reprogramming alters genome organization and promotes establishment of a totipotent chromatin “ground state”. This project is led by a strong multi-disciplinary team bringing together expertise in polymer physics (Mirny), ovary organoids (Saitou), biochemistry and single-molecule imaging (Peters) and zygote biology (Tachibana-Konwalski).

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Title: Defying the reproduction-maintenance trade-off: Role of diet in long-lived termite reproductives

Abstract: We propose to investigate how dietary restrictions (DR) affect aging from a new angle by using social insects as models. Aging is a hallmark of most bilateria and most animals balance their reproduction rate against lifespan. Intriguingly, this trade-off is inverted in reproductive individuals (queens) of social insects (termites, ants, bees). Whereas most studies on aging directly manipulate the lifespan, e.g. of mice or worms or other lab-bred animals, we here propose a radically new approach by employing easily accessible and natural extremely long-lived termite queens as models. Their metabolism, response to DR and fertility will be gauged against genomically identical bu infertile and short lived workers, as well as shorter lived and less fecund queen of a closely related termite species. We will sample termite colonies directly from the field, keep them, expose them to DR and measure their fitness and fecundity. We will examine the role of DR during colony development by sampling transcriptomes, analyzing their epigenetic status, their metabolome and endocrine status and performing in-depth molecular analyses of key molecular components that are known to be implicated in regulating aging and fecundity. Using multiple OMICS methods, reverse genetics, hormonal and dietary administration we will be able to disentangle pathways involved in development of queens and measure the impact of energetic metabolic reprogramming on fitness and reproduction status. Expression patterns and spatio-temporal changes of genetic networks will be used to develop a simple state model. In this model, the metabolic status can be used to predict an individual's trajectory of aging and fecundity depending on its epigenetically imprinted background such as it's caste. Our project thus establishes a new model system for studying the relationship between DR, aging and fecundity, in which the latter two are decoupled and comparison of our model to other model organisms will help understand which dependencies and molecular components have universally conserved interaction partners or phenotypic effects. The project is possible only due to the four participants from three continents, with expertise in dietary research, energy metabolism, field research and social insect genomics.

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Title: Disentangling trophic and sexual transmission dynamics in a ubiquitous parasite

Abstract: Toxoplasma is a common parasite of birds, rodents, and humans during the asexual phase of its life cycle. But, having sex for Toxoplasma is limited to cat intestines. It enters cat when an infected rat or bird is eaten up. This creates an interesting problem because rats do not prefer to be eaten up. Toxoplasma blocks the fear of cats from infected rats. This is often taken to mean that infected rats will approach cat without fear and be eaten up at higher rates. We have no direct evidence for this indirect assumption. We will test this in the first part of this project. Kangaroo Island in Australia plans to fence off one of its parts and exterminate all cats from there. The island is doing so to protect wildlife and to reduce Toxoplasmosis in livestock. From our point of view, this is a golden opportunity to test if Toxoplasma increases predation of rats by cats. We can now compare the survival of infected rats and mice vis-à-vis uninfected rats and mice in the same landscape before and after cat removal. We expect to see more infected animals dying than uninfected when cats are around; and no difference when cats are removed.

While having sex is limited to cats, Toxoplasma can still sustain its asexual life cycle through carnivory or sexual reproduction. The sexual reproduction is exciting because such infections usually have less virulence. They do not kill a host very often as dead hosts do not reproduce to further transmit the parasite. Parasites moving across the food chain, from rats to cats for example, do not have such limitation and often increase virulence. But what happens when an infection is simultaneously transmitted by sexual and going-up-food chain mode? Both paths must trade-off with each other. When cats are plentiful it pays to increase predation (by reducing fear in infected rats) even if it means a bit less of sexual transmission and vice versa. This is a difficult assumption to test directly. The situation in Dudley Peninsula provides a perfect opportunity. We will collect Toxoplasma from bodies of infected rats and mice before and after cat extermination. We will bring these bugs in the lab and ask if cat removal means that the parasite now concentrates more on sexual transmission. That would mean a robust invasion of testes, more pheromones and more liking elicited from uninfected females. This is the second part of this project.

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Title: How cerebrospinal fluid physico-chemical properties impact body axis formation and scoliosis

Abstract: Organ development depends on the integration of local cell-to-cell interactions with long range signalling throughout the body. We will investigate mechanisms by which long-range signalling via the cerebrospinal fluid (CSF) regulate body axis formation and spine curvature. The CSF is produced by the choroid plexus in the brain ventricles and flows down the central canal in the spinal cord. The CSF instructs brain development by delivering age-dependent grow-promoting factors to target cells. CSF circulation also contributes to the curvature of body axis (embryo) and to the spine (juveniles). However, the mechanisms regulating the flow and content of CSF remain poorly understood. Progress has been impeded by a historical lack of tools and challenges inherent to studying fluids in small, developing organisms. The advent of innovative tools and approaches now provides an unprecedented opportunity to overcome previous limitations. The transparency of zebrafish and the accessibility to mouse CSF provide powerful models for testing our driving hypothesis that the physical and biochemical properties of CSF impact body axis formation and spine curvature. We propose an entirely new, interdisciplinary collaboration of three leaders in their field: (1) physicist Francois Gallaire (EPFL, Switzerland), expert in the theory & modelling of complex fluid dynamics; (2) developmental neurobiologist Maria Lehtinen (Boston Children's, HMS, USA), expert in analysis of the choroid plexus-CSF system; (3) biophysicist Claire Wyart (ICM, France), expert in imaging and sensory physiology in the spinal cord. Our multi-tiered approach will unravel the principal parameters driving CSF composition and flow. We will map CSF flow in the developing fourth ventricle and central canal (Aim 1), elucidate mechanisms regulating protein secretion into the CSF (Aim 2), and investigate mechanisms of active transport of instructive signals along the anteroposterior axis controlling organogenesis (Aim 3). The proposed studies will transform this historically understudied area of neuroscience into a robust field spanning CSF-based signalling in brain and spine. As studies of paracrine signalling and fluid dynamics lag far behind cell-intrinsic studies of signalling, our techniques and concepts should provide a roadmap for future studies of fluid niches throughout the vertebrate body.

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Title: Detecting inequity in dendritic cells through bio-inspired synthetic T cells

Abstract: During infection with bacteria or viruses, our immune system becomes activated to fight these foreign invaders and thereby prevent us from getting ill. Dendritic cells are an important immune cell type that control this process. When they sense the presence of infection, they trigger an immune response against any simultaneously captured bacterial or viral molecules by displaying the molecules on their surface for immune cells to recognise. However, they can also capture normal "self" molecules from our organs during infection, and it is unclear how they focus the immune response upon the foreign invaders without triggering an inappropriate response against "self" molecules that could inflict damage upon our organs. As we currently have only a limited ability to detect and visualise self and foreign molecules on the dendritic cell surface during an infection, our understanding of this process is incomplete.

We propose a bio-engineering approach to address this problem. Nature has already designed a system for biological molecule detection on the dendritic cell surface: immune cells called T cells are exquisitely sensitive at recognising specific surface molecules on dendritic cells. We will leverage the key features of this interaction to develop novel staining materials that imitate the natural way that T cells recognize molecules on dendritic cells. Using an engineering technology based on DNA origami to control the size, shape, and function of small particles, a "synthetic T cell" staining reagent will be assembled on a DNA scaffold. By adding fluorescent signals and experimenting with a variety of engineering blueprints, we can increase the sensitivity and signal intensity of our particles. Combining these synthetic T-cells with state-of-the-art microscopy techniques that can image individual molecules, we will precisely pin-point the exact location of self and foreign molecules on the DC surface during infection to determine if their organisation helps to either inhibit or promote an immune response. Overall, this project will thus employ expertise from a range of different disciplines to dissect how the immune system discriminates between "self" and "foreign" molecules.

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Title: Evolutionary puzzles: Do microbes in the Atacama Desert harvest UV as an energy source?

Abstract: Melanin is a unique molecule synthesized by all domains of life (bacteria, archaea, and eukarya), but it is most commonly known as a UV screening pigment in humans and other animals. However, surprisingly little is known about the secondary or tertiary roles of the molecule. The recent discovery that melanin-containing fungi located at the ruptured Chernobyl reactor display enhanced growth in the presence of gamma radiation, which is usually thought to be harmful, has hinted at the diverse functions of the molecule. A defining feature of melanin is the chemical and structural variability found across and within microbial species. This feature along with the ability to broadly absorb over the UV and visible spectrum has led to the hypothesis that microbial cells could have evolved to capture and utilize this energy. Low resource environments such as those found in deserts are the most probable habitats in which these adaptations could exist. The main objective of this proposal is to assemble a diverse team of scientists to apply new single cell and single molecule technology to studying divergent biology in one of the most extreme environments on the planet, the Atacama Desert.

Here we test the hypothesis that melanin is a key player in producing biochemical energy in microorganisms that are exposed to high amounts of radiation in the UV range. As part of the project, our international team, consisting of engineers, biochemists and microbiologists, will carry out a comprehensive analysis of the microbes living in the Atacama Desert, one of earth's most UV-irradiated regions. We will identify alternate functions of melanin using state-of-art technologies to 1) sort out and identify melanin producers; 2) sequence their DNA for melanin pathway analysis; 3) characterize how melanin is being synthesized; and 4) link melanin to the photochemical conversion of UV light. Since microbial communities and melanin represent mixed populations, technology is needed which can probe individual cells and molecules. Only once this is accomplished, can sub-populations within each group be thoroughly examined. The impact of understanding melanin not only extends the fundamental knowledge of a commonplace pigment, but also can also prove useful for searching for life on other UV-irradiated environments such those on other planets.

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Title: Tracing AID/APOBEC- and MSI-mediated hyper-mutagenesis in the clonal evolution of gastric cancer

Abstract: Cancer evolves through the continuous acquisition of DNA mutations. As a result, multiple diverse tumor subclones can arise during the course or tumor progression from benign to malignant carcinoma. These diverse cancer subclones have different traits, such as different levels of therapy resistance or metastatic potential. Intriguingly, cancers can obtain a hypermutator phenotype that leads to accelerated acquisition of DNA mutations. In gastric cancers, a medical burden especially in East Asia, multiple types of mutations are observed, suggestive of pronounced impact of hypermutator phenotypes on the development of these cancers. However, how hypermutator action facilitates the evolutionary trajectory of cancers is not fully understood.

In this HFSP project, we intend to use organoid technology to study the impact of diverse hypermutator phenotypes in gastric cancers. Organoid technology is a state-of-the-art culture technique for human mini-organs in a dish from both normal tissues as well as cancers, and mimics the in vivo scenario to great extent.

To understand the role of hypermutator action in human gastric cancer, three research teams integrate their expertise into a novel experimental pipeline: 1) the establishment and analysis of patient-derived tumor organoids with natural occurring hypermutator phenotypes, in parallel with engineered tumor organoids with introduced hypermutator phenotypes. 2) Monitoring mutational accumulation within these tumor organoids using DNA sequencing at every intermediate step along their progression towards malignant carcinoma and 3) filming the diverging cellular behaviors between different tumor subclones using advanced microscopy. Ultimately, we aim to map the dynamic mutation landscape during the evolutionary trajectory of diverging tumor subclones in comparison to the phenotype of the changing tumor cells.

This project will provide a unique opportunity to obtain a comprehensive understanding on the role of hypermutator phenotypes in cancer evolution. Moreover, we expect that our improved insights on the emergence of genetic subclones in gastric cancer can guide us to understand to other cancer subtypes and will help us to fight against chemo resistance and metastasis of cancer.

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Title: Behavior-dependent optimization of the brain's metrics for space and time

Abstract: Is our perception of time and distance the same when we walk in the park, travel in a high speed train, or wait for the starting gun in a field race? Over a century ago, Einstein opened the way into considering space and time as relative variables. New evidence now suggests that areas of our brain might have been doing so all along. In this case, however, rather than resulting from the gravitational pull of a massive star, the effect would be the natural consequence of a simple principle of economy. Not all dimensions need to be represented all the time; one could possibly do better with simplified schemas adapted to every situation in an information compressed manner.
The entorhinal cortex is an area of the brain where a rodent or human represents its own position in space and time. Recent evidence suggests that spatio-temporal dimensions can actually be represented in a flexible way, depending on behavioral demands. We lack, however, all knowledge about the repertoire of possible schemas that it can choose from, on how these schemas are formed and stored, and on the neural mechanisms behind the selection process. We here propose to study the neural basis of this "brain relativity" in rodents by means of a variety of tools that include behavioral experiments, recordings of neural activity, manipulation of specific neural circuits and computational modeling. We will challenge animals by making them explore artificial environments with geometries that require a switch between representations, as would happen when touring through an Escher scene. A room will be slowly morphed into a corridor, or distance estimation into time. We hope that this will allow us to understand the shape that these representations have in the brain, their number, and the mechanism that controls which one is applied at any given time. We also plan to identify specific circuits of the rodent entorhinal cortex that encode information about space and time using state-of-the art genetic tools, which will allow us to gain on-line control over the selection of schemas and its behavioral consequences. Along the process, results will be compared with computational models to understand the logic underlying each observation. All this will help us to get a deeper insight into how we perceive and interact with space and time in our daily experience.

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Title: Molecular control of cortical homeostasis and cell polarization

Abstract: The cell cortex is a dynamic network of actin filaments (F-actin), myosin motors, and accessory proteins coupled to the cell membrane, which mediates both the maintenance and changes of animal cell shape. Cell shape is determined by a balance between resistance to mechanical perturbation and the mechanical stresses produced internally by the actomyosin cytoskeleton. The precise architecture of the F-actin network and the dynamics of F-actin assembly and disassembly determine the accumulation of myosin-generated stresses, resulting in diverse behaviors from orchestrating local pulsatile contractions to inducing cell-scale polarised flows. For instance, in xenopus oocytes and embryonic cells of echinoderms and frogs, positioning of the actomyosin cytokinetic ring is preceded by travelling waves of F-actin assembly and disassembly1. Similarly, local pulsatile actomyosin contractions lead to persistent constriction within the drosophila mesoderm2, or global polarization and flow of the cortex of the developing C. elegans embryo3. Thus, contractility often follows the maintenance of a dynamic but steady cytoskeleton. However, how the spatial and temporal balance of actomyosin contractility, F-actin architecture and biochemical signaling determine homeostasis or polarized flows in the cytoskeleton remains an outstanding question in molecular cell biology.

Here we propose a unique integration of ex vivo, in vitro and in silico studies to uncover the molecular design principles for cell polarization. Ex vivo, we will modulate protein-protein interactions and use optogenetic control of Rho and light inactivation control of myosin to determine the extent to which cortical stability entrains force generation to impact polarization in neutrophils. Guided by such experiments, we will spatiotemporally modulate protein-protein interactions within an in vitro model cytoskeleton to alter F-actin architecture, adhesion, and myosin activity. In silico, we will systematically vary these parameters to generate testable predictions for cortical stability versus polarization. We envisage that our integrated approach will decipher the general principles underlying the assembly, stability and dynamics of cytoskeletal structures and the regulation of eukaryotic cell shapes.

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Title: Remembering the future: Interactions between sensation, memory, and behavior

Abstract: Memory is typically treated as representing past experiences, including salient events and stimuli. A key purpose for the retention of these memories is to provide predictive information that guide the animal's future behavior - where it will go and what it will do. However, the world is rarely static, and memory therefore requires continuous updating from sensory information. While sensation and memory have been well studied separately in tightly constrained tasks, little is known about how they interact to enable complex behaviors. This proposal aims to understand how these three processes – behavior, sensation, and memory – predict each other while animals freely move within an environment, and how these predictions are altered based on an animal's experience and the predictability of its environment.

We will test three general hypotheses about the interactions between sensation, memory and behavior: (1) Memory evolves from being learnt from (i.e. instructed by) sensation and behavior in a novel environment, to being predictive of (i.e. instructing) sensation and behavior in a familiar environment. (2) The direction of predictability between processes (i.e. which process instructs another) are dynamic and can be manipulated by changing the context of the environment. (3) The direction of predictability is dependent on the predictability of the environment, with memory instructing sensation and behavior for predictable features, but sensation instructing the other processes for unpredictable features.

We will test these hypotheses in an environment with static (predictable) and mobile (unpredictable) features. We will combine long-term recordings from large populations of neurons in the visual cortex and the hippocampus of freely moving animals, high-resolution behavioral measurements, and statistical modeling of neural and behavioral dynamics. Our experiments will provide unprecedented measurements of the neural representations that underlie natural behavior. They will show how these animals, and potentially ourselves, use representations of the past to predict the future.

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Title: Visual circuit adaptations to natural environments and behaviors in zebrafish and cichlids

Abstract: How does an animal perceive the world and execute the appropriate behavior? This task requires sensory organs such as the eye to detect features of the environment, then for the brain to integrate this information and produce a pattern of motion directed at a goal. However there is currently little information about how animals encode features from their natural environment. Indeed, most of our understanding about visual neural processing in the fish brain comes from the use of artificial stimuli that lack the complexity found in the natural visual environment. Fish are ideal for investigating visual processing because their diverse behaviors and compact brains are amenable to modern neuroscience technologies. How does the brain process information? Most current understanding of fish vision relies on the common lab model zebrafish (Danio rerio), but other species have likely evolved distinct solutions for visual processing based on environmental and behavioral differences. To bridge these knowledge gaps, we propose to thoroughly characterize the natural visual environments of two species, zebrafish and Astatotilapia burtoni (an African cichlid), by collecting a large dataset of natural underwater videos in India (zebrafish) and Lake Tanganyika, Zambia (cichlid). We will then use these videos in laboratory experiments with a custom-built immersive viewing arena to examine which visual features from the natural environment elicit behavioral responses in the two fish species. To examine brain responses to natural stimuli, we will transfer existing technology for recording brain activity in larval zebrafish to cichlids. We hypothesize that there will be species-specific brain function adaptations underlying several behavioral differences between these species in response to visual motion. We will then expand from studying basic visual features to analyzing the appearance of other fish during social behavior. What drives fish to school or fight? How does the surrounding environment influence how fish detect the presence of conspecifics and other fish? Our integrative and comparative approach will provide a novel perspective on how vertebrate visual brain areas evolved to encode and adapt to the natural world.

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Title: Active morphological colloids for probing and tailoring intracellular antigen processing

Abstract: Dendritic cells, a type of white blood cell, are the sentinels of the immune system. Upon infection with a pathogenic microbe, such as a bacteria or fungus, dendritic cells can ingest the microbe by a process call phagocytosis. Dendritic cells then kill the ingested microbe by huge amounts of hydrogen peroxide, a disinfectant, and subsequently degrade the microbe by enzymes. This releases protein fragments from the microbes which can be presented to T cells, another type of white blood cell. T cells thereby learn to recognize the microbe and can mount a pathogen-specific immune response.

Dendritic cells need to be able to ingest many different types of microbes with a large variation in motilities, sizes and shapes, ranging from very small viruses to fungal hyphens. Although it is well-known that many types of pathogens use their shape and motility to try to avert immune responses, the underlying cell biological mechanisms are still largely unknown. The goal of this project is to address how microbe shape and motility affect their processing by dendritic cells for T cell activation.

We plan to chemically engineer bacteria-sized artificial particles with well-defined but highly irregular shapes, such as star-shaped particles. These particles will be equipped with chemical engines that are fuelled by hydrogen peroxide (produced by the dendritic cells) to generate motion. We expect that the shape and forces exerted by these particles will affect their transport and processing within the crowded environment of dendritic cells. The behaviour of these particles will be studied in well-defined dense suspensions that mimic the complex environment within dendritic cells. These biophysical experiments will be combined with experiments with dendritic cells isolated from the blood of healthy volunteers. This will allow a full quantitative understanding of how pathogen shape and motility affect their fate in dendritic cells.

This study will lead to a better understanding of how pathogens use their shape and motilities to avoid clearance by the immune system. Moreover, understanding how microbe shape and motilities affect immune responses will allow us to generate particles that elicit very fast and strong immune responses or, conversely, no (or very slow) immune responses, which will aid in the design of improved vaccines and carriers for drug delivery.